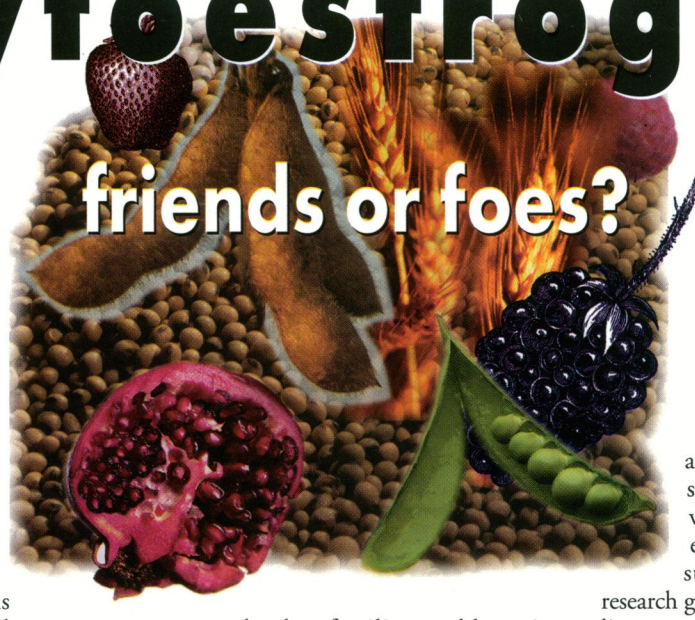
Phytoestrogens

friends or foes?



Phytoestrogens are plant chemicals that may act as fungicides, deter herbivores, regulate plant hormones, and protect plants against ultraviolet radiation. Structurally, some phytoestrogens resemble endogenous estrogens of humans and animals, and recent research suggests they may also function as estrogen agonists or antagonists when eaten by humans. Although humans have used phytoestrogens medicinally for thousands of years, only in the last 15 years or so have researchers begun to look beyond the folk remedies to investigate phytoestrogens' possible roles in modern health care. Although the popular media has at times heralded phytoestrogens as panaceas, medical data remain inconclusive. Still, recent epidemiological studies and experiments with animals suggest many varied benefits of phytoestrogens. "Although much indepth research has been done to identify and characterize the pharmacokinetics of certain phytoestrogens, the whole area of using phytoestrogens for medicinal purposes remains wide open," says Retha Newbold, a research biologist in the NIEHS Reproductive Toxicology Group.

Reports about environmental estrogens, or xenoestrogens, have been widespread in the last few years. There are important distinctions between estrogenic compounds of industrial origin and those that come from plants, however. Compounds such as the insecticide DDT and industrial PCBs have been implicated by some researchers in causing estrogen-dependent cancers in exposed populations. These same compounds have also been suggested as causes of declining sperm counts and other fertility problems in humans, as well as reproductive failures and anatomical abnormalities in wildlife. Unlike some industrial xenoestrogens, which tend to bioaccumulate in adipose tissue and persist in the body for years, phytoestrogens are readily metabolized and spend relatively little time in the body. However, during this time they can have significant effects on body systems. The timing of exposure, repeated exposures, and levels of exposure to phytoestrogens are important. "The issue is a lot more complicated than it looks on the surface," says Newbold. "Just because a substance, like phytoestrogens, is naturally produced doesn't make it automatically harmless or beneficial."

How They Work

Extensive research has been done to identify the types of phytoestrogens that are found in humans and how they are metabolized. The two major classes of phytoestrogens that have captured the most scientific attention are lignans and isoflavones. Kenneth Setchell, associate professor of pediatrics at the University of Cincinnati Children's Hospital, began studying phytoestrogens in the early 1980s. For several years Setchell and his colleagues had been puzzled by unknown steroids in biological fluids. As part of a study on hormonal fluctuations in women, he began investigating urinary steroid hormone metabolites. A closer look at these compounds prompted speculation that they might be previously unidentified endogenous estrogens. Therefore, it came as a surprise when two independent research groups identified the compounds as lignans, which weren't known to exist in humans. The two lignans, named enterolactone and enterodiol, are actually the products of microbial metabolism of secoisolariciresinol and matairesinol, compounds found in whole grains, fibers, and flax seeds, as well as several fruits and vegetables. Enterodiol may be further oxidized to enterolactone. All four of the lignans may be absorbed from the gut.

Isoflavones, which are abundant in legumes, have also been identified in human biological fluids. Within plant tissues, isoflavones exist as sugar derivatives called glycosides, and concentrations vary widely depending on stressors such as viral, bacterial, fungal, or herbivore attack. These compounds undergo hydrolysis in the human gut, yielding aglycones. Like the lignans, these aglycones meet one of three fates: they may be excreted or absorbed from the gut, or undergo further metabolism. The four most common isoflavones are formononetin, daidzein, genistein, and biochanin A. Biochanin A is metabolized to genistein. If it is not absorbed into the body, it may be further metabolized to *p*-ethylphenol, a hormonally-inert compound. Formononetin may be metabolized to daidzein, which is further metabolized mostly to equol, a more potent phytoestrogen, and to O-desmethylangolensin. Products of both lignan and isoflavone metabolism may be excreted or absorbed. If absorbed, the phytoestrogens undergo

conjugation in the liver with glucuronic acid, or to a lesser extent, sulfate, and are excreted in the urine or in the bile. Some intestinal bacteria produce β-glucuronidases, enzymes that can deconjugate phytoestrogen metabolites when they pass through the intestine, setting the stage for their recirculation through the body.

Nonsteroidal plant estrogens were first identified in the early 1930s, with the discovery that soybeans, willows, dates, and pomegranates contain compounds with structural similarity to estrogens. It was unknown whether these compounds could have biological activity in animals until a concurrent discovery was made in phytoestrogens' effects on Australian sheep. Female sheep were plagued by reproductive system lesions and sharp declines in fertility. Animal husbandry experts linked the problem to the sheep's grazing on *Trifolium subterraneum*, a species of clover. Researchers identified the clover compounds equol and coumestrol (another phytoestrogen) as being responsible for the sheep's reproductive problems. Once the etiology of clover disease had been established, scientists began to question whether these compounds also affected other species. Equol and other phytoestrogens such as enterolactone and enterodiol were discovered in human biological fluids in concentrations as much as 5,000 times greater than endogenous estrogens. The question then became whether these compounds presented a reproductive or other risk to humans or whether their presence might be in some way beneficial. "Unless you ask what is the quantitative risk [of phytoestrogens]," says Michael Bolger, a toxicologist at the FDA's Center for Food Safety and Applied Nutrition, "how can you integrate it with the benefits to get the full picture?"

Benefits

Scientists have begun to piece together the full picture of phytoestrogens by looking at populations who consume them the most. Asian populations consume a diet that is very rich in the phytoestrogens genistein and daidzein, which are found in soybeans and soy products. These phytoestrogens occur at levels of 50–300 milligrams per 100 grams in soy beans, and in lower levels in soy products such as miso, soy milk, and tofu.

NIEHS

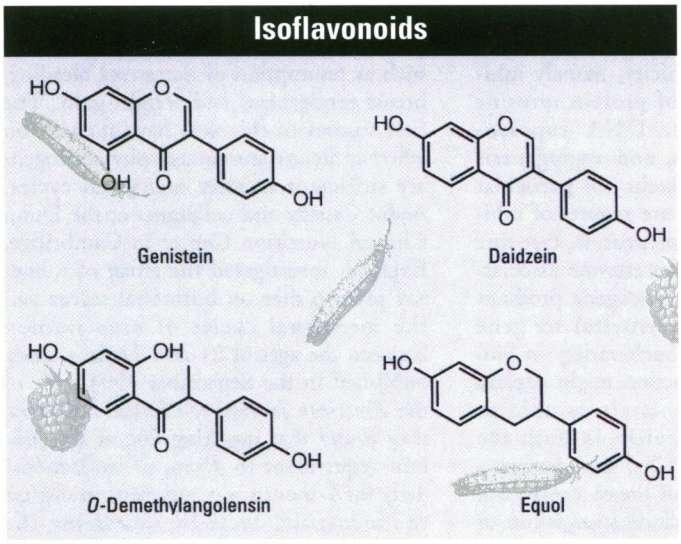
Retha Newbold—Naturally-produced substances are not automatically harmless or beneficial.

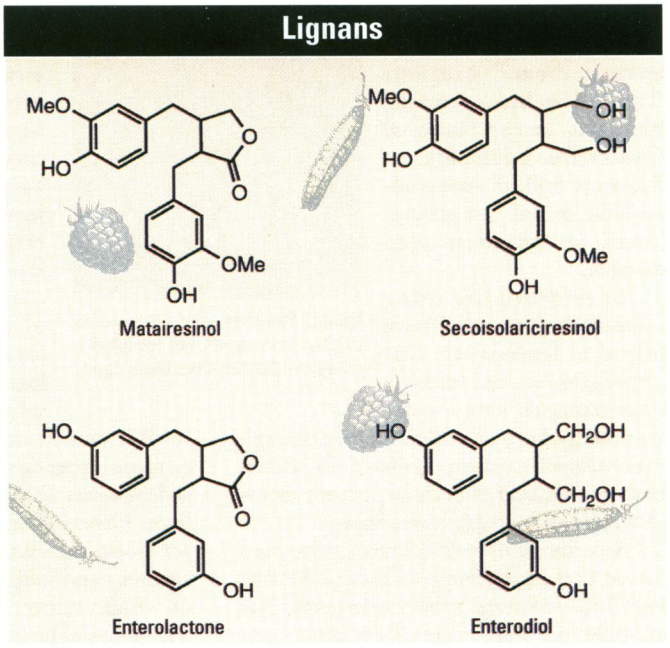
Asian populations also suffer a significantly lower rate of hormone-dependent cancers compared to westerners. They also have a much lower incidence of other hormonally-associated problems such as osteoporosis and menopausal symptoms. The presence of phytoestrogens in Asian diets and the comparatively low rates of diseases prevalent in western populations—including breast, endometrial, prostate, and colon cancers, as well as coronary heart disease—suggests that phytoestrogens may have protective effects.

Studies of immigrants have bolstered the argument that different disease rates between eastern and western populations may spring more from diet than from other factors, such as genetics. Although the genetic link to several cancers has been well established, the genetic propensity to develop cancer does not vary significantly between eastern and western populations. For example, Japanese men develop small latent prostatic carcinomas at the same rate as western men, although their mortality from prostate cancer is much lower. However, Asian immigrants to western countries tend to alter their diets to the typical western diet that includes more protein and fat, less fiber, and fewer soy products. As their diets change, their risks for certain hormonally-related diseases increase.

These effects may be due to certain properties of phytoestrogens. For example, lignans are associated with the fiber portion of seeds and grains. Because fiber increases fecal bulk and decreases intestinal β-glucuronidase levels, it reduces the circulation of conjugated estrogens in the liver and intestines. By indirectly reducing the amount of bioavailable hormone, fiber may reduce cancer risk.

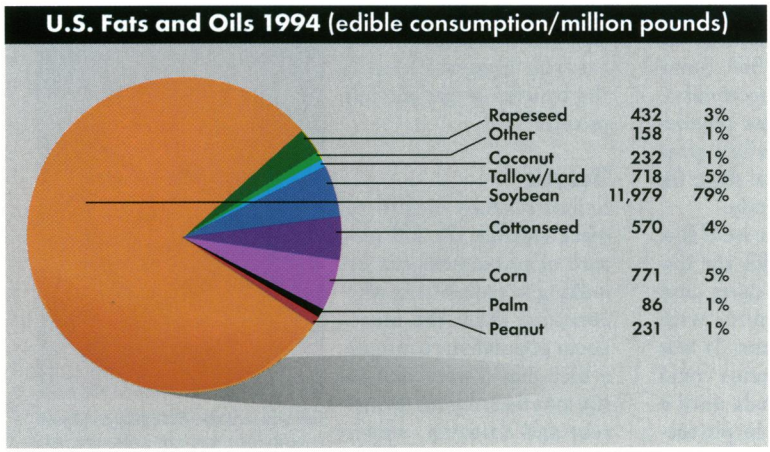
Other research has focused on the effects of a lack of beneficial dietary factors rather than the presence of detrimental components. Herman Adlercreutz, professor and chairman of the Department of Clinical Chemistry at the University of Helsinki, reviewed phytoestrogens in articles in the October 1995 *EHP Supplement* and (with colleagues) the March 1995 supplement of the *Journal of Nutrition*. Adlercreutz present-





Puzzle pieces. The structures of some of the most important phytoestrogens for humans have been identified. Source: Adlercreutz H. Phytoestrogens: epidemiology and a possible role in cancer protection. *Environ Health Perspect Suppl, 103(7):103–112 (1995).*

ed evidence from many studies which shows that lignan and isoflavonoid excretion rates correlate with dietary and population groups. People who ate a macrobiotic diet and vegetarians had significantly higher urinary excretion rates of lignans compared to meat eaters and subjects with breast cancer. Low urinary lignan values were also seen in Japanese men and women, consistent with their low consumption of whole grain products. This group's high consumption of soy products was reflected in their high urinary and plasma concentrations of isoflavonoids. Metabolites of phytoestrogens in biological fluids indicated that people who consumed phytoestrogens in their diets also metabolized and absorbed them.

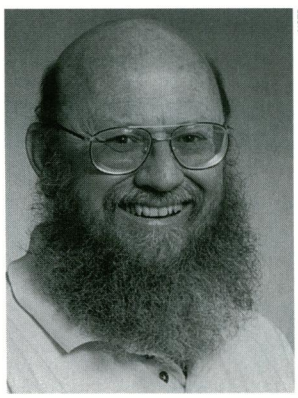


Soy surprise. Americans may be reaping the benefits of soybeans by eating more of them than they think.

Results showing that variations exist among dietary and population groups with regard to plasma, urinary, and fecal concentrations of estrogens and estrogen metabolites provide some evidence that phytoestrogens affect sex hormone metabolism. How these differences relate to disease rates is being investigated.

In Vitro and Animal Studies

Many of the studies that provide evidence for the benefits of phytoestrogens were conducted for the purpose of investigating other endpoints and, therefore, are not accepted as definitive. "These studies add weight to the questions, but they don't answer them," cautions Daniel Sheehan, research biologist, at the National Center for Toxicological Research. Still, *in vitro* studies have served as a springboard for phytoestrogen research.


NCTR

Daniel Sheehan—While benefits of phytoestrogens are intriguing, no safety studies have been done.

In vitro studies using radiolabelled estradiol have helped to demonstrate that phytoestrogens and endogenous estrogens have a common mechanism of action, namely through the estrogen receptor. It has been shown that phytoestrogens can elicit both estrogenic and antiestrogenic responses.

According to Newbold, most researchers accept that phytoestrogens such as lignans and isoflavones are weakly estrogenic. For example, the equilibrium dissociation constant for genistein is 100 to 10,000 times greater than for estradiol or DES, which means that genistein's ability to stay bound to an estrogen receptor is less than one-hundredth that of the more potent estrogens. Furthermore, if genistein binds with an estrogen receptor, it elicits less than one-thousandth the response of an endogenous estrogen.

In vitro data have demonstrated that phytoestrogens may inhibit cell cancer growth. For example, using the MCF-7 human breast cancer cell line, which depends on estrogen for proliferation, researchers have shown that the lignan enterolactone inhibits proliferation in the presence of estradiol, a stronger estrogen. Alone, enterolactone stimulates proliferation.

In vitro data have also demonstrated biological activity by phytoestrogens that is not associated with estrogenicity, namely inhibition of protein tyrosine kinases, DNA topoisomerases, and angiogenesis by genistein. Of particular interest are reports of inhibition of protein tyrosine kinase, an enzyme associated with oncogene products of the retroviral *src* gene family. Such variety in biological action might explain why genistein is able to inhibit cancer cell growth in both the estrogen-dependent MCF-7 and estrogen-independent MDA-468 breast cancer cell lines. Other effects include stimulation of sex hormone–binding globulin (SHBG) synthesis and inhibition of aromatase, both of which indirectly affect the amount of free steroidal hormones in the body. Also, some evidence exists for inhibition of 17β-hydroxysteroid oxidoreductase type I, the enzyme responsible for reversible conversion of 17β-estradiol to estrone. In addition to the inhibiting cancer cell growth, genistein has also been shown to induce differentiation of cells into mature phenotypes.

The *in vitro* studies have provided the basis for *in vivo* studies of phytoestrogens. Most such *in vivo* studies have been conducted in rats and mice, but some have been conducted on nonhuman primates. These studies highlight the difficulties in extrapolating from *in vitro* results to whole systems. For example, in a 1994 study in *Anticancer Research*, oncologist Harmesh R. Naik and colleagues at Wayne State University School of Medicine compared *in vitro* and *in vivo* data on genistein's ability to affect hormone refractory prostate cancer. Although genistein was cytotoxic in both rat and human prostate cancer cell lines, it failed to inhibit proliferation of implanted prostate cells in rats.

Experimental Studies in Humans

Researchers are becoming fascinated by phytoestrogens' potential as cancer preventatives and as nonpharmaceutical interventions for menopausal symptoms and osteoporosis. Current estrogen replacement therapies treat menopausal symptoms and may help to prevent health problems such as breast and endometrial cancers and osteoporosis. Many women are reluctant to take estrogenic drugs because of side effects such as resumption of menstrual bleeding, breast tenderness, and weight gain. The first studies in this area have focused on whether dietary amounts of phytoestrogens are sufficient to alter menstrual cycles. Aedin Cassidy and colleagues at the Dunn Clinical Nutrition Center in Cambridge, England, investigated the effect of a high soy-protein diet on hormonal status and the menstrual cycles of nine women between the ages of 21 and 29. In a study published in the September 1994 issue of the *American Journal of Clinical Nutrition*, they found that ingesting 60g of soy protein (equivalent to 45mg of isoflavones) daily for 1 month was sufficient to disrupt the menstrual cycle by increasing the length of the follicular phase and delaying the onset of menstruation. Flax seed, a rich source of lignans, has also been demonstrated to induce cycle changes.

Results of some studies related to possible anticancer effects of isoflavonoids and lignans

Type of cancer	Compound or food	Species or cell type	Effect or result
Breast cancer	Genistein	MCF-7 cells	Competition with estradiol
Breast cancer	Diet and phytoestrogen excretion	Women	Low urinary excretion in women at higher risk
Breast cancer	Soy bean chips	Rat	Inhibition of tumor growth
Breast cancer	Flaxseed	Rat	Protective
Breast cancer	Genistein, biochanin A	MCF-7 + other cells	Inhibition of proliferation
Breast cancer	Enterolactone	MCF-7 cells	Inhibition of proliferation in the presence of estradiol
Colon cancer	Soy intake	Japanese men, women	Reduced risk
Colon cancer	Flaxseed	Rat	Protective
Gastric cancer	Genistein	HGC-27 cells	Growth inhibition
Gastric, esophagus, colon cancer	Genistein, biochanin A	Many types of cells	Inhibition of proliferation
Liver cancer	Genistein	HepG2 cells	Inhibition of proliferation
Liver cancer	Enterolactone	HepG2 cells	Stimulation of SHBG synthesis
Prostate cancer	Soy products	Men of Japanese ancestry	Less risk
Prostatitis	Soy food	Rat	Preventive effect
Prostatic dysplasia	Soy food	Male mice	Inhibition
Leukemia	Genistein	MOLT-4, HL-60 human cells	Inhibits cell cycle, progression, and growth
Leukemia	Genistein	Human HL-60, K562 cells	Induction of differentiation
Myeloid leukemia	Daidzein	HL-60 cells	Induction of differentiation
Melanoma	Genistein	5 cell lines	Induction of differentiation
Embryonal carcinoma	Genistein	Mouse F9 Cells	Induction of differentiation
Solid pediatric tumors	Genistein	Neuroblastoma, sarcoma	Inhibition of proliferation
Placental microsomes	Lignans	Human	Inhibition of aromatase
Endothelial cells	Genistein	Many different endothelial cells	Inhibition of angiogenesis

Source: Adlercreutz H, Phytoestrogens: Epidemiology and a possible role in cancer protection. *Environ Health Perspect Suppl, 103(7):103–112 (1995).*

Experiments with menopausal women have been less conclusive. Epidemiologist Donna Baird and colleagues at the NIEHS looked at a variety of measurements in an attempt to determine the estrogenicity of dietary soy in postmenopausal women in a study published in the May 1995 issue of the *Journal of Clinical Endocrinology and Metabolism.* After consuming a soy diet including 165 mg of isoflavones daily for one month, the subjects showed very little estrogenic response: levels of follicle-stimulating hormone, luteinizing hormone, and SHBG did not change significantly. There was a small effect on the maturation of the vaginal epithelium.

Setchell, who was involved with the study, speculated that more effect might have been shown if the experiment had lasted longer, citing an Australian study that ran for three months. This study, which appeared in the April 1995 issue of *Maturitas*, was conducted by Alice L. Murkies, a doctor of medicine at the Brighton Medical Clinic in Victoria, Australia. Murkies and colleagues measured hot flashes as a determinant of estrogenic activity in response to phytoestrogen-rich diets. This experiment found significant reductions in the occurrence of hot flashes when the diets of postmenopausal women were supplemented with soy or wheat flours for 12 weeks. Of the test diets, soy seemed to yield the best results, but the authors did not indicate the amounts of actual phytoestrogens the test subjects consumed on a daily basis. Neither soy nor lignans have been examined for protective effects against osteoporosis. However, ipriflavone, a phytoestrogen similar to genistein, has been shown to stimulate osteoblasts.

Human studies on hormones and diet may be confounded by a number of largely uncontrollable variables including genetics, individual intestinal flora, transit time, effects of medicines, health, and hormonal status. Many of the diseases being studied for phytoestrogenic effects are also multifactorial. Researchers have indicated that a prospective study is needed to tease out phytoestrogens' effects.

Risks

Although studies on phytoestrogenic benefits seem promising, researchers have voiced concerns that consumption of large amounts of phytoestrogens may cause adverse health effects, especially with regard to development and fertility. "My position is that, while we are intrigued by the possible benefits, the fact of the matter is that no safety studies, especially with regard to development, have been done," says Sheehan. "Estrogens are clearly a two-edged sword in humans." Newbold also expresses concern that the effects of developmental exposure to phytoestrogens is simply not known. "We are not sure for adults if the 'natural' doses are helpful, or at least not harmful." Also, the possible interactions of phytoestrogens with established medical treatments such as estrogen replacement therapy or cancer therapy are unknown.

Neonatal and *in utero* exposures to sex steroids regulate the development of sexually differentiated behavior, reproductive physiology, and central nervous system anatomy and neurochemistry. In a letter published in the 24 May 1995 issue of the *New Zealand Medical Journal*, Cliff Irvine, a professor of animal and veterinary science, and colleagues at Lincoln University in New Zealand indicated that soy-based infant formulas in New Zealand contain 3–5 times as much daidzein and genistein as the amount that will disrupt a woman's menstrual cycle. Considering diethylstilbestrol's estrogenic effects on development, Irvine stated that this exposure should be investigated. Setchell says that infants metabolize the phytoestrogens, but how these compounds act in their bodies is unknown. One point of view is that they might negatively affect development, while others believe developmental effects would be negligible, and that exposure might actually help ward off hormone-related ill health in the future.

Patricia Whitten, an associate professor of anthropology, and colleagues at Emory University have investigated possible developmental effects of coumestrol. Results of the study, published in the March 1995 supplement to the *Journal of Nutrition*, showed that in neonatal and immature rats, endogenous estrogens were low, and that coumestrol induced estrogenic responses, including premature estrous cycles. In normal adult female rats, coumestrol proved antagonistic to the endogenous estrogens and disrupted the ovarian cycle. Whitten and colleagues have also found that male and female neonatal rats exposed to coumestrol via their mothers' milk had altered numbers of progestin receptors in the pituitary and hypothalamus, as well as altered sexual behavior and gonadotropin function. These results led researchers to conclude that coumestrol has a negative effect on neuroendocrine devel-

opment. Although coumestrol has shown fertility and developmental effects, it's difficult to extrapolate these results to humans, whose exposure to coumestrol is dwarfed by their exposures to genistein, daidzein, and lignans.

Very little research has examined genistein's effect on development outcomes. Claude Hughes, now an associate professor of comparative medicine and obstetrics and gynecology at the Bowman Gray School of Medicine at Wake Forest University, examined neonatal exposure to genistein while working at Duke University Medical Center. Hughes and colleague Jill R. Levy in Duke's Department of Obstetrics and Gynecology found that exposure to genistein disrupted secretion of luteinizing hormone by the pituitary gland. In a recent paper published in the January 1995 issue of the *Proceedings of the Society for Experimental Biology and Medicine*, Levy and colleagues revealed that *in utero* exposure of rats to genistein may decrease markers such as birth weight and anogenital distance, and in female rats may delay the onset of puberty. Though these results do not confirm any risks for human infants, they do not discount them either.

Coral Lamartiniere, a professor of pharmacology and toxicology at the University of Alabama, and other researchers offer the argument that early exposure to genistein might actually be beneficial. In one study, published in the January 1995 issue of the *Proceedings of the Society for Experimental Biology and Medicine*, Lamartiniere and colleagues injected neonatal rats with genistein. Once the rats reached maturity, they were exposed to dimethylbenz[*a*]anthracene to induce mammary carcinogenesis. Rats that had been exposed to genistein as neonates had significantly increased latency in developing tumors, as well as reduced incidence of tumors. Lamartiniere said similar chemopreventive results were observed in rats exposed to genistein during puberty only. Despite lengthening their estrous cycles, their follicular development, sex steroid concentrations, and fertility seemed fine. "Common sense would tell us that soy does not pose a problem for fertility," said Setchell, pointing to the reproductive success of Asians. However, he added, that fact could be countered with other similarly logical arguments. One such argument, according to Hughes, is that Asians have been consuming these diets for centuries, and any soy-related fertility problems may have been selectively bred out of the population generations ago. In that case, westerners suddenly switching to a soy-based diet might not have the advantage of that natural selection. Sheehan also adds that, especially with developmental toxicants, there is a long latency period, which makes it difficult to associate an event with a negative outcome. "The fact that there aren't any negative reports can't be taken as an argument that soy diets are safe," he said.

The current knowledge base on phytoestrogens fuels speculation and arguments, but doesn't yield definitive answers. For all the varying opinions about phytoestrogens, however, researchers are united in the call for more definitive research. "What seems eye-opening to me is that we are looking at the development of a field," says Newbold. "It's just now come to the forefront."

Julia Barrett